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(54) VACCINE COMPOSITIONS FOR MUCOSAL DELIVERY

IMPFSTOFFZUSAMMENSETZUNGEN FUER MUKOSALE ABGABE

COMPOSITIONS DE VACCIN A ADMINISTRATION PAR LA MUQUEUSE

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(56) References cited:
EP-A- 0 209 281
EP-A- 0 462 534
EP-A- 0 484 621
WO-A-90/13313
WO-A-90/15871
WO-A-92/15689

- VACCINE vol. 10, no. 1, 1992, GUILDFORD,UK pages 53 - 60 CHATFIELD ET AL 'CONSTRUCTION OF A GENETICALLY DEFINED SALMONELLA TYPHI TY2 AROA,AROC MUTANT FOR THE ENGINEERING OF A CANDIDATE ORAL TYPHOID-TETANUS VACCINE'
- INFECTION AND IMMUNITY vol. 58, no. 5, 1990, WASHINGTON D.C.,USA pages 1323 - 1326 FAIRWEATHER ET AL 'ORAL VACCINATION OF MICE AGAINST TETANUS BY USE OF A LIVE ATTENUATED SALMONELLA CARRIER'
- DATABASE WPI Section Ch, Week 9135, Derwent Publications Ltd., London, GB; Class B04, AN 91-260180
- DATABASE WPI Section Ch, Week 8807, D rwent Publications Ltd., London, GB; Class B04, AN 88-045809

Descripti n

[0001] The present invention relates to acellular vaccine compositions for intranasal delivery to mucosal surfaces.

[0002] More particularly, the present invention relates to acellular intranasal vaccine compositions for inoculating a meal such as a human against tetanus infections.

[0003] It has long been the practice of clinicians to immunise human infants against a variety of common diseases by means of mixed vaccines which are directed against a plurality of diseases. For example, multiple-component vaccine compositions directed against diphtheria, tetanus and whooping cough have been available for a considerable number of years. Such vaccines have hitherto been administered by injection. The advantages of multiple-component vaccines are readily apparent in that the patient (usually an infant) is subjected to a much smaller number of potentially distressing injections than would otherwise be the case.

[0004] The majority of infectious diseases are initiated by contact with a mucosal surface. The infecting agent may remain at or within the mucous membranes during the course of the infection or may penetrate into the body and localise at other sites. The importance of the mucous membranes in the first line of defence against infectious disease can be gleaned from the fact that 90% of the lymphocytes of the body underlie such surfaces. Priming mucosal surfaces by immunisation so that they respond vigorously and effectively control pathogenic organisms they encounter would be advantageous. Unfortunately traditional immunisation regimes are ineffective at eliciting mucosal responses. The systemic and local (mucosal) immune systems appear to be compartmentalised and in general do not impinge on one another; that is parenteral immunisation with non-living vaccines stimulates mucosal immune responses weakly if at all. Mucosal immunisation (oral or intranasal) can evoke serum antibodies but thin is usually less effective than parenteral immunisation. The immunocytes of the different mucous membranes form a vast intercommunicating network, termed the common mucosal immune system, such that topical immunisation of one surface (e.g. the gastrointestinal tract) may lead to an immune response at that surface and also distance surfaces such as the respiratory tract.

[0005] EP-A-0209281 suggests that vaccine compositions including a tetanus toxin protein or peptide can be administered orally, but the Examples in this document only relate to administration by injection.

[0006] International Application WO-A-9015871 discloses a process for the production of tetanus toxin C fragment and discloses also that a vaccine composition containing C fragment can be administered *inter alia* orally or parenterally, but only administration by injection is specifically exemplified.

[0007] S.N. Chatfield *et al*, Vaccine Vol. 10, Issue 1, 1992 pp. 53-60 describe the construction and use as a vaccine of a mutant form of salmonella which incorporates in its chromosome a gene for the non-toxic 50kD fragment of tetanus toxin. Although oral administration of the vaccine is mentioned, again, no experimental data are provided.

[0008] Fairweather *et al*, Infection and Immunity, May 1990 pp. 1323-1326 disclose the oral delivery of a salmonella strain incorporating a gene for tetanus toxin C fragment. Also disclosed is the administration of tetanus toxin fragment C via the subcutaneous route, but no reference is made to the oral administration of fragment C *per se*, or to the administration of fragment C by the intranasal route.

[0009] EP-A-462534 describes acellular anti-pertussis vaccines comprising a double mutant of pertussis toxin, filamentous haemagglutinin and the 69kD protein, alone or in combination with diphtheria and tetanus anatoxin. However, there is no reference in the document to the oral administration of the vaccine compositions, nor is there any reference to intranasal administration.

[0010] WO-A-9013313 describes a synergistic combination of B.pertussis FHA and 69kD antigen and suggests that such vaccines may be administered rally, but there are no examples in this document of th oral administration of such antigens. Moreover, there is no disclosure of intranasal administration.

[0011] JP-A-63002932 discloses generically the administration of peptides by the intranasal route in combination with a specified absorption enhancer.

[0012] Manclark and Shahin (US Patent application number 07/532 327, filed 5.6.90 - available through the US Department of Commerce, National Technical Information Service, Springfield, VA 22161, USA) - have described the intranasal and intraduodenal administration of filamentous hemagglutinin (FHA) obtained from Bordetella pertussis and have illustrated that FHA is an effective mucosal immunogen. Manclark and Shahin speculated in USSN 07/532 327

is the ability of these molecules to bind to mucosal epithelial cells via certain surface receptors, although it has been found in studies by others that there is not necessarily a correlation between the ability of an antigen to bind to eucaryotic cells and its mucosal immunogenicity.

[0014] Thus, as far as we are aware, there is currently no way of predicting with any certainty whether a given antigen will possess good mucosal immunogenicity.

[0015] We have now found that certain molecules make excellent mucosal immunogens and such components can be utilised in the development of a mucosally (intranasally or orally) delivered vaccine against the diseases whooping cough and tetanus. In particular, we have found that the P69 outer membrane protein (P69 - also known as pertactin) from B.pertussis and the non-toxic immunogenic 50Kd portion of tetanus toxin (C-Fragment) from C.tetanii are highly immnogenic when given intranasally. C-Fragment and P.69 were generated by DNA recombinant technology. Recombinant C-Fragment and P.69 produced from E.coli and yeast have been demonstrated to be immunogenic and protective in mice, see M.Roberts et al, Recombinant P.69/pertactin: immunogenicity and protection of mice against Bordetella pertussis infection; Vaccine 10, 43 (1992); and see also N.F. Fairweather et al, Infection and Immunity, 55, 2541 (1987).

[0016] In a first aspect, the invention provides the use of an acellular antigen (hereinafter referred as "(a)"), which is a mucosally immunogenically active substance comprising the 50kD C fragment of tetanus toxin, an immunogenic fragment thereof, or a derivative thereof formed by amino acid deletion, substitution or insertion, for the manufacture of an acellular vaccine composition for intranasal administration to a mucosal surface to induce an immune response in the mucosal surface against tetanus infection.

[0017] There is also described the use of a mixture of antigens for the manufacture of an acellular vaccine composition for intranasal administration to a mucosal surface to induce an immune response in the mucosal surface against each of the said antigens, the mixture of antigens comprising:

(a) a mucosally immunogenically active substance comprising the 50kD C fragment of tetanus toxin, an immunogenic fragment thereof, or a derivative thereof formed by amino acid deletion, substitution or insertion; and
(b) a mucosally immunogenically active substance comprising the P.69 outer membrane protein of B.pertussis; an immunogenic fragment thereof, or a derivative thereof formed by amino acid deletion, substitution or insertion.

There is also described the use of a mixture of antigens as hereinbefore defined but wherein said mixture comprises in addition to (a) and (b);

(c) a mucosally immunogenically active substance comprising B.pertussis filamentous haemaglutinin, an immunogenic fragment thereof, or a derivative thereof formed by amino acid deletion, substitution or insertion.

[0018] In a further aspect, the invention provides an acellular vaccine composition in a form adapted for intranasal application to a mucosal surface, the composition comprising antigen (a) or a mixture of antigens as hereinbefore defined and a pharmaceutically acceptable carrier.

[0019] The compositions of the present invention are formulated for delivery to the respiratory (e.g. nasal and bronchial) mucosa.

[0020] Typically the compositions are formulated as an aqueous solution for administration as an aerosol or nasal drops, or as a dry powder, e.g. for inhalation.

[0021] Compositions for administration as nasal drops may contain one or more excipients of the type usually included in such compositions, for exampl preservatives, viscosity adjusting agents, tonicity adjusting agents, buffering agents and the like.

[0022] The antigens may take the form of the tetanus toxin C fragment per se, th P.69 protein per se, or the B.pertussis haemaglutinin per se. Or it may take th form of a larger molecule containing on or more of the aforesaid antigens, immunogenically active fragments thereof, or derivatives formed by amin acid deletion, substitution and insertion, provided that the larger molecul is immunogenically activ when administ red directly to the mucosa.

[0023] The antigen or mixture of antigens typically is selected such that it is non-toxic to a recipient thereof at concentrations employed to elicit an immune response.

[0024] Two mor of the antigens forming the mixture may he presented in a singl molecule. Such a molecule may he prepared by recombinant methods by preparing a DNA construct containing genes coding for two or more of the

or E.coli heat-labile toxin, genetically detoxified variants thereof or their binding (B) sub-units in the vaccine.

[0027] The vaccine composition may optionally contain another mucosally immunogenically active portion of the tetanus toxin molecule. In addition, the mixed vaccine may contain one or more further mucosally immunogenically active antigens.

[0028] The vaccine composition may, in addition to non-toxic immunogenic forms of tetanus toxin and pertussis antigens, contain non-toxic immunogenic forms of diphtheria toxin and immunogenic forms of Haemophilus influenzae group B polysaccharide (HiB), thereby providing a mucosal diphtheria-tetanus-pertussis (DTP) vaccine or DTPHiB vaccine.

[0029] The P.69 outer membrane protein of B.pertussis is a protein of approximately 61 KD molecular weight; see A.J.Makoff et al, "Protective surface antigen P.69 of Bordetella pertussis: its characteristics and very high level expression in Escherichia coli", Bio-Technology, 8, 1030 (1990).

[0030] It can be prepared and isolated according to the method disclosed in P.Novotny et al: The Journal of Infectious Diseases, 164, 114 (1991), or recombinant material prepared from E.coli by the method given in the article by A.J.Makoff et al referred to above. It can bind to eukaryotic cells.

[0031] Purified B.pertussis filamentous haemaglutinin usually contains polypeptides of differing molecular weight ranging from 98-220 KD, and can be isolated and purified from cell culture supernatants of B.pertussis, for example as described in the article by P. Novotny et al referred to above. The filamentous haemaglutinin is able to bind to eukaryotic cells and cause haemaglutination of sheep erythrocytes.

[0032] The C fragment of tetanus toxin is a peptide of approximately 50KD molecular weight which can be isolated and purified from E.coli by the method described in A.J.Makoff et al, Bio/Technology, 7, 1043 (1989). The C fragment is characterised by an ability to bind the eukaryotic cells possessing the trisialoganlioside G_T1b and by an ability to elicit protection in mice against lethal challenge with tetanus toxin.

[0033] The antigenic molecules of the present invention can be prepared by isolation and purification from the organisms in which they occur naturally, or they may be prepared by recombinant techniques and expressed in a suitable host such as E.coli in known manner. When prepared by a recombinant method or by synthesis, one or more insertions, deletions, inversions or substitutions of the amino acids constituting the peptide may be made.

[0034] Each of the aforementioned antigens is preferably used in the substantially pure state. The quantity of the mixture of antigens administered will depend, in part, upon the purity of the individual antigens. Thus, for a substantially pure form of the P.69 outer membrane protein, a dose in the range from about 1-100 microgrammes/dose typically would be administered to a human, the actual amount depending on the immunogenicity of the preparation in humans when applied to mucosal surfaces.

[0035] For a substantially pure form of the B.pertussis filamentous haemaglutinin, and the 50KD C fragment of tetanus toxin, a typical dose range would be of the order given above in respect of P.69 protein. In a typical immunisation regime employing the antigenic preparations of the present invention, the vaccine may be administered in several doses (e.g. 1-4), each dose containing 1-100 microgrammes of each antigen. The immunisation regime may involve immunisation purely by the mucosal route or by a combination of mucosal and parenteral immunisation. The dosage will in general depend upon the immunogenicity of the different antigens when applied to the respiratory or gastrointestinal tracts of humans.

[0036] The invention will now be illustrated in greater detail by reference to the specific embodiments described in the following examples.

[0037] The examples are intended to be purely illustrative of the invention and are not intended to limit its scope in any way.

BRIEF DESCRIPTION OF THE FIGURES

[0038]

Figure 1 illustrates the growth of B.pertussis in the lungs of intranasally immunised mice. Mice wer immunised intranasally with two doses of antigen on day 0 and day 29 and then aerosol challenged with B.pertussis 14 days later (day 43). The counts represent the means of 4 pairs of lungs ± SEM.

Figure 2 illustrates th local immune response in the lungs of mic in response to intranasal immunisation and challenge. Lymphocytes were purified from th lungs of groups of mic after intranasal immunisation and aerosol challenge and the number of cells secreting antibody to the immunising antigen was determined by the ELISPOT assay. Counts represent the response of lymphocyt s pooled from 4 mic .

Figure 3 illustrates th primary antibody response to pertussis antigens in th lungs of mice following aer sol challenge. Lymphocytes from Lungs removed 10 days after aerosol challenge were assayed for antibody production against P69 or FHA. Mice immunised with one antigen wer tested against th other antigen. Counts repr s nt the response of lymphocytes pooled from groups of 4

EXAMPLE 1 PERTUSSIS COMPONENTS

[0039] Mice were immunised with 12μg of either P.69, FHA or OVA on day 1 and day 29. Mice were then aerosol challenged with Bordetella pertussis on day 43 (14d post boost). Lungs were removed from groups of mice at periods after challenge, homogenised and viable bacterial counts performed to determine the growth of B.pertussis in the respiratory tract. The results are shown in Figure 1. Bacteria increased in numbers in the lungs of control mice (OVA) during the first seven days. In contrast, in both P.69 and FHA immunised animals bacterial number declined during this period and at the day 7 time point the levels of bacteria in the lungs of immunised mice were only 1-4% of those in the control group. By day 14 bacteria had almost cleared from the lungs of the immunised mice but were still present in large numbers in the OVA immunised mice.

[0040] The local immune response in the lungs of mice was analysed by enumerating cells secreting antibody against FHA, P.69 or OVA amongst lymphocytes isolated from the lungs of mice by "ELISPOT". There were low numbers of antibody secreting cells (ASC) producing antibody against the immunising antigens after the second (Figure 2). Following aerosol challenge of ASC in the lungs of mice immunised with FHA or P.69, but not OVA, increased by several orders of magnitude (Figure 2). Cells secreting antibody of IgG, IgA and IgM isotypes were detected.

[0041] The increase in ASC following aerosol challenge may represent a primary response to the antigens present in the challenge organisms or a boost of the response in immunized mice. To see which of these options was correct, lung lymphocytes were screened against antigens which the mice had not encountered until challenge (FHA immunised mice were tested against P.69 etc). As can be seen from Figure 3, the responses were similar in all mice and comparisons with the same time point (d23) in Figure 1 shows that prior intranasal immunizations boost the response several fold.

[0042] The serum response was studied using ELISA and the results are shown in Table 1 below:

Table 1

Serum anti-pertussis antibody response of intranasally immunised mice						
	ANTIBODY TITRE[a]					
	Days post-immunisation					
	8		20		24	
Group	FHA	P.69	FHA	P.69	FHA	P.69
FHA	<50	<50	400	100	1600	100
P.69	<50	<50	<50	200	200	400
OVA	<50	<50	<50	<50	100	200

[a] Determined by Elisa

[b] Mice aerosol challenged d 14

[0043] There were no detectable antibodies against FHA of P.69 prior to aerosol challenge (d8). There were no anti-OVA antibodies to serum of OVA immunised mice (data not shown). Following challenge (>d14) the anti-FHA response did exhibit large increase in FHA immunised mice. This increase was specific, the anti-P.69 response did not increase proportionally in these mice. There was a small increase in anti-P.69 titre in P.69 immunised mice compared to FHA or

Table 2

Protection of mice against tetanus toxin challenge by I/N immunisation with C fragment						
Group	Dose	No of Mice	Survival post-challenge			
			Day			
			2	3	4	5
1. C-frag	20µg	10	3	3	2	1
2. C-frag	2 x 20µg	10	10	8	8	8
3. Ova	2 x 20µg	10	0	0	0	0

SERUM RESPONSE

[0045] The mice receiving 2 doses of C fragment had a mean serum anti-C fragment titre of greater than 200 prior to challenge.

MATERIALS AND METHODS

ANTIGENS

[0046] P. 69 was synthesized intracellularly in E.coli and purified as described in A.J.Makoff et al, Bio/Technology 8, 1030 (1990). FHA was provided by SKB under and exchange of reagents agreement. C fragment was produced from E.coli as in A.J.Makoff et al, Bio/Technology 7, 1043 (1989). Antigens were diluted in PBS immediately prior to immunisation.

IMMUNISATION

[0047] Adult (6-8 weeks) BALB/c mice were anaesthetised with metathane. 30µl of antigen solution was added to the external nares of mice (15µl/nares) as they recovered consciousness. Antigen was taken into respiratory tract by inhalation.

AEROSOL CHALLENGE WITH B.PERTUSSIS

[0048] Mice were placed in cages on a rotating carousel in a plastic exposure chamber as described in P.Novotny et al. Development for Biological Standards, 61, 27 91985). A bacterial suspension in PBS was prepared from 2-to 3-day old cultures of B.pertussis BBC26 grown on CW blood agar plates. The mice were exposed to an aerosol (generated from the bacterial suspension) of 2 x 10^9 Colony-forming units (CFU) in PBS by a Turret mouthpiece tubing operated by a System 22 CR60 high-glow compressor (Medic-Aid), Pagham, W st Sussex, UK) giving a very fine mist at a dynamic flaw of 8.5 litres/min. The generated mist was drawn through a chamber by a vacuum pump at a passage of ca.12L of air per mist mixture per min, which maintained 70% relative humidity in the chamber. The exposure to aerosol lasted 30 min; a period of 10 min then allowed the chamber to clear.

[0049] The course of the infection was assessed by performing counts f viabl bacteria in lungs. Groups of four mice were removed at intervals and killed by cervical dislocation, and their lungs were aseptically removed and homogenised in a Potter Elvehjem homogenizer with 2ml of PBS. Dilutions of the homogenate were spotted onto Cohen Wheeler

sorbent assay (ELISA). Antigen (50µl; 1 g/ml in phosphate-buffered saline, pH7.2) was absorbed onto 96-well microtitre plates (EIA 'Costar' NBL, Northumbria, UK) by incubation at 4°C overnight. Wells were aspirated and washed three times with PBS containing 0.05% (v/v) Tween 20 (PBS-T; Sigma), and then blocked with 3% (w/v) bovine serum albumin (BSA; Sigma) in PBS. After washing, 50µl of test serum appropriately diluted in PBS-T-0.1% BSA was added per well. After incubation at 37°C for 2h, the wells were washed and incubated at room temperature with 50µl of substrate (0.04% 0-phenylenediamine hydrochloride; Sigma) dissolved in phosphate citrate buffer (pH5.0[24mM citrate, 64mM disodium hydrogen phosphate] containing 40µl hydrogen peroxide). The reaction was terminated by the addition of 50µl 1M sulphuric acid. Plates were read in a Titertek Multiscan MCC ELISA reader at 492nm.

[0052] The titre was expressed as the reciprocal of the highest dilution of test serum that gave an absorbance reading twice that of the similarly diluted pre-bleed serum. Absorbance values below 0.1 were discarded.

ELSIPOT Assay for specific antibody secreting cells (ASC) in murine lungs.

[0053] Local antibody production in the murine lung was determined using the ELISPOT technique. Lymphocytes were isolated from murine lungs as follows: Lungs were washed briefly in PBS to remove traces of blood and then were finely chopped with a scalpel blade. 1ml of PBS containing 10mM $MgC1_2$, 0.5U/ml collagenase A (Boehringer Mannheim, Lewes, UK) and 0.25mg/ml DNase 1 (Boehringer) was added for each pair of lungs and incubated at 37°C with gentle agitation for 45 min. The mixture was then passed through a 40 gauge mesh. Lumps were pressed through the mesh with the plunger from a 5ml syringe. The cell suspension was placed in a centrifuge tube and allowed to stand for several minutes to allow large debris to settle. The supernatant was removed and the cells were pelleted and washed several times. Red cells and non-viable cells were removed by centrifugation on a Ficol-Isopaque gradient (LSM,Flow Laboratories Ltd, Hertz, UK). After washing cell viability was determined by Trypan Blue exclusion. Cells were finally suspended in RPM11640 complete Medium (10% foetal calf serum, penicillin 100IU/ml, streptomycin 100 g/ml, L-glutamine 2mm; Flow).

[0054] The ELSIPOT assay was performed as follows. Briefly, 24-well tissue culture plates (Costar) were coated overnight with P.69, FHA or OVA (0.5ml of 1 g.ml in PBA) after washing and blocking 0.5ml volumes of dilutions of the lymphocyte suspension in complete RPM_1 1640 were added to the wells and incubated at 37°C/10% CO_2 for 3h. After washing goat anti-mouse IgG, A or M (1/1000, Sigma) and rabbit anti-goat IgG-alkaline phospbatase (1/1000, Sigma) were added sequentially. Finally, substrate solution (0.5µl of 1mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in 2-amino-2-methyl-1,3-propanediol (AMP) buffer, Sigma) was added and plates were incubated until blue spots were visible under low power microscopy.

Claims

1. The use of an acellular antigen, (hereinafter referred to as "(a)"), which is a mucosally immunogenically active substance comprising the 50kD C fragment of tetanus toxin, an immunogenic fragment thereof, or a derivative thereof formed by amino acid deletion, substitution or insertion for the manufacture of an acellular vaccine composition for intranasal administration to a mucosal surface to induce an immune response in the mucosal surface against tetanus infection.

2. An acellular vaccine composition in a form adapted for intranasal application to a mucosal surface, the composition comprising the mucosally immunogenically active substance (a) as defined in Claim 1, and a pharmaceutically acceptable carrier therefor.

3. A vaccine composition according to Claim 2 in the form of a solution for administration as a nasal spray or nasal drops.

4. A vaccine composition according to Claim 3 in the form of nasal drops, said composition comprising means for administering drops to the nasal mucosa.

Patentansprüche

1. Verwendung eines azellulären Antigens (im folgenden als „(a)" bezeichnet), welches eine auf die Schleimhaut bezogene immunogen aktive Substanz ist, umfassend das 50 kD C-Fragment des Tetanustoxins, ein immunogenes Fragment davon oder ein Derivat davon, gebildet durch Aminosäure-Deletion, -Substitution oder -Insertion zur Herstellung einer azellulären Vakzinezusammensetzung für die intranasal Verabreichung an eine Schleimhautoberfläche zur Auslösung einer Immunantwort in der Schleimhautoberfläche auf eine T tanusinfektion.

2. Azelluläre Vakzinezusammensetzung in einer Form, die an die intranasale Verabreichung an die Schleimhautoberfläche angepaßt ist, wobei die Zusammensetzung die auf die Schleimhaut bezogene immunogen aktive Substanz (a), wie in Anspruch 1 definiert, und einen pharmazeutisch geeigneten Träger dafür umfaßt.

3. Vakzinezusammensetzung nach Anspruch 2 in Form einer Lösung zur Verabreichung als Nasenspray oder Nasentropfen.

4. Vakzinezusammensetzung nach Anspruch 3 in Form von Nasentropfen, wobei die Zusammensetzung Mittel zur Verabreichung der Tropfen an die Nasenschleimhaut umfaßt.

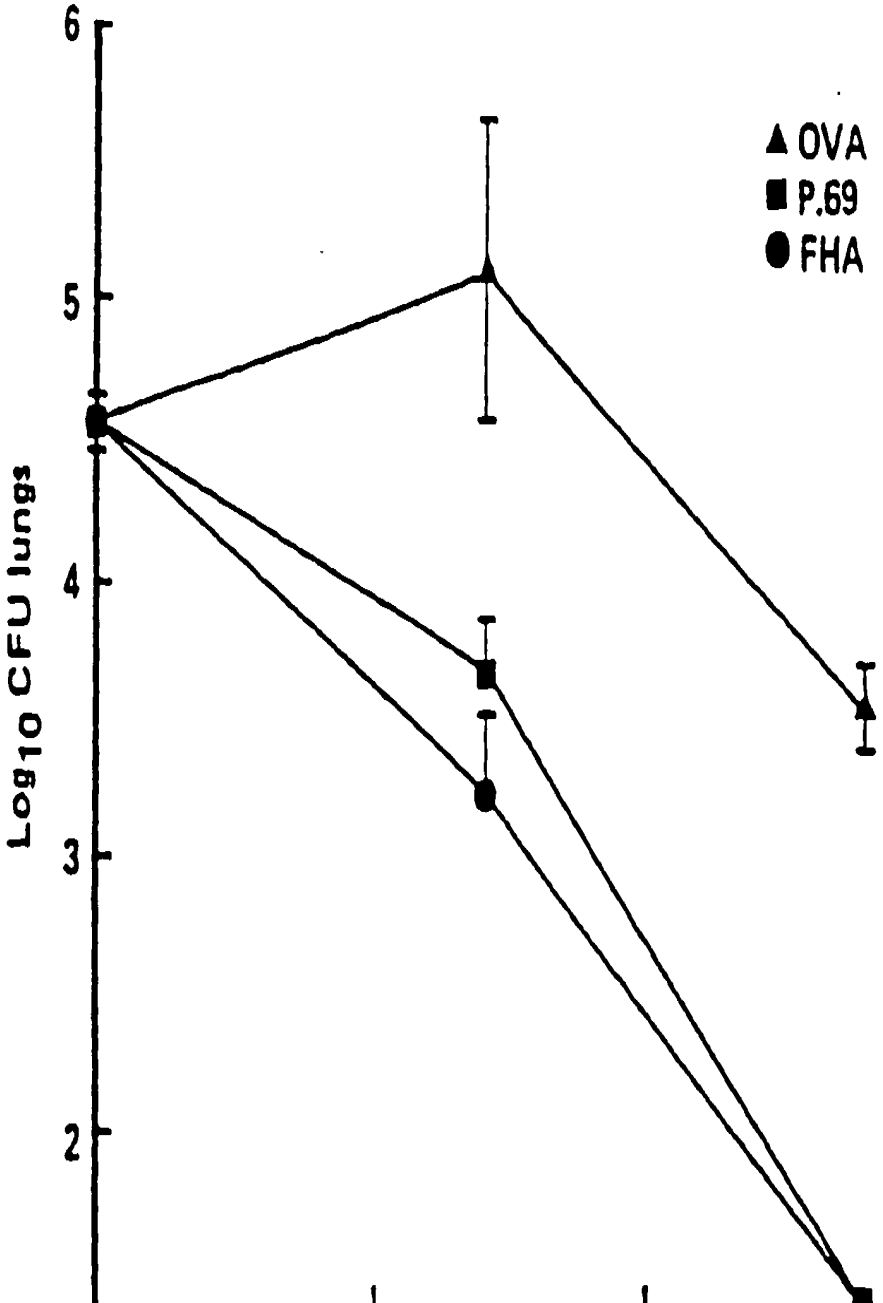
Revendications

1. Utilisation d'un antigène acellulaire, (désigné ci-après par "(a)"), qui est une substance douée d'activité immunogène par voie muqueuse comprenant le fragment C de 50 kDa de la toxine tétanique, un fragment immunogène de celui-ci, ou un dérivé de celui-ci formé par suppression, substitution ou insertion d'acides aminés pour la fabrication d'une composition vaccinale acellulaire pour administration à une surface muqueuse par voie intra-nasale en vue d'induire une réponse immunitaire dans la surface muqueuse contre une infection tétanique.

2. Composition vaccinale acellulaire sous forme appropriée à l'application à une surface muqueuse par voie intranasale, la composition comprenant la substance douée d'activité immunogène par voie muqueuse (a) telle que définie dans la revendication 1, et un véhicule pharmaceutiquement acceptable pour cette application.

3. Composition vaccinale selon la revendication 2 sous forme d'une solution pour administration sous forme d'aérosol nasal ou de gouttes nasales.

4. Composition vaccinale selon la revendication 3 sous forme de gouttes nasales, ladite composition comprenant des moyens pour l'administration de gouttes à la muqueuse nasale.


6
5
4
3
2
Log10 CFU lungs
▲ OVA
■ P.69
● FHA

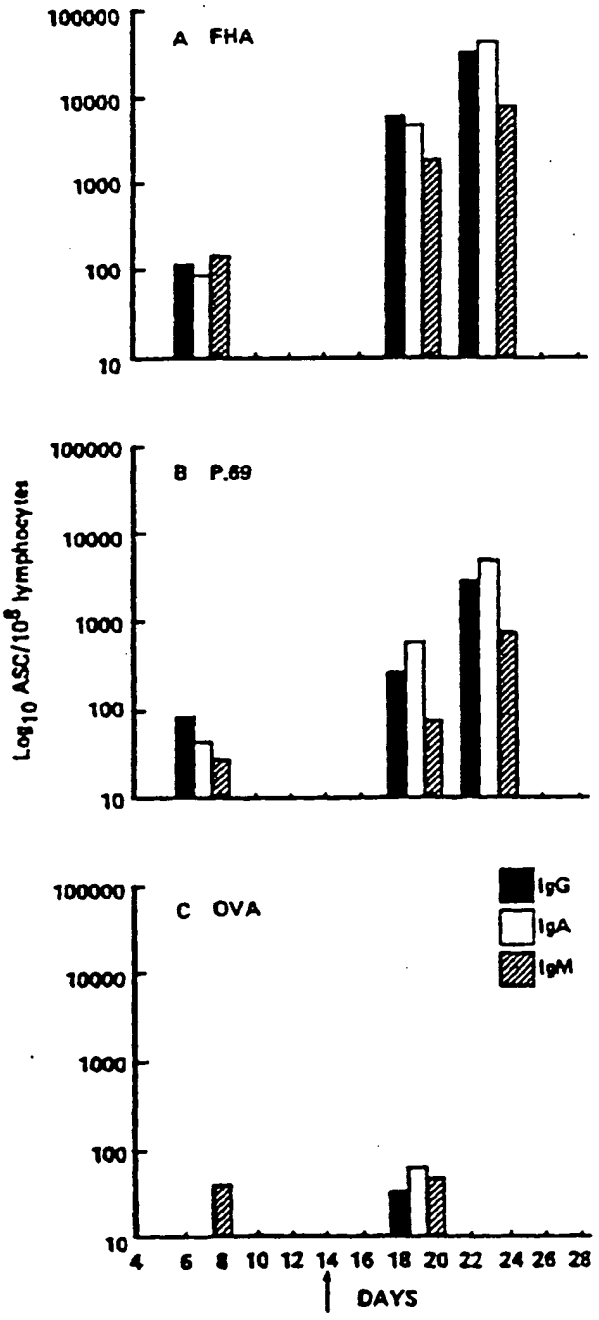
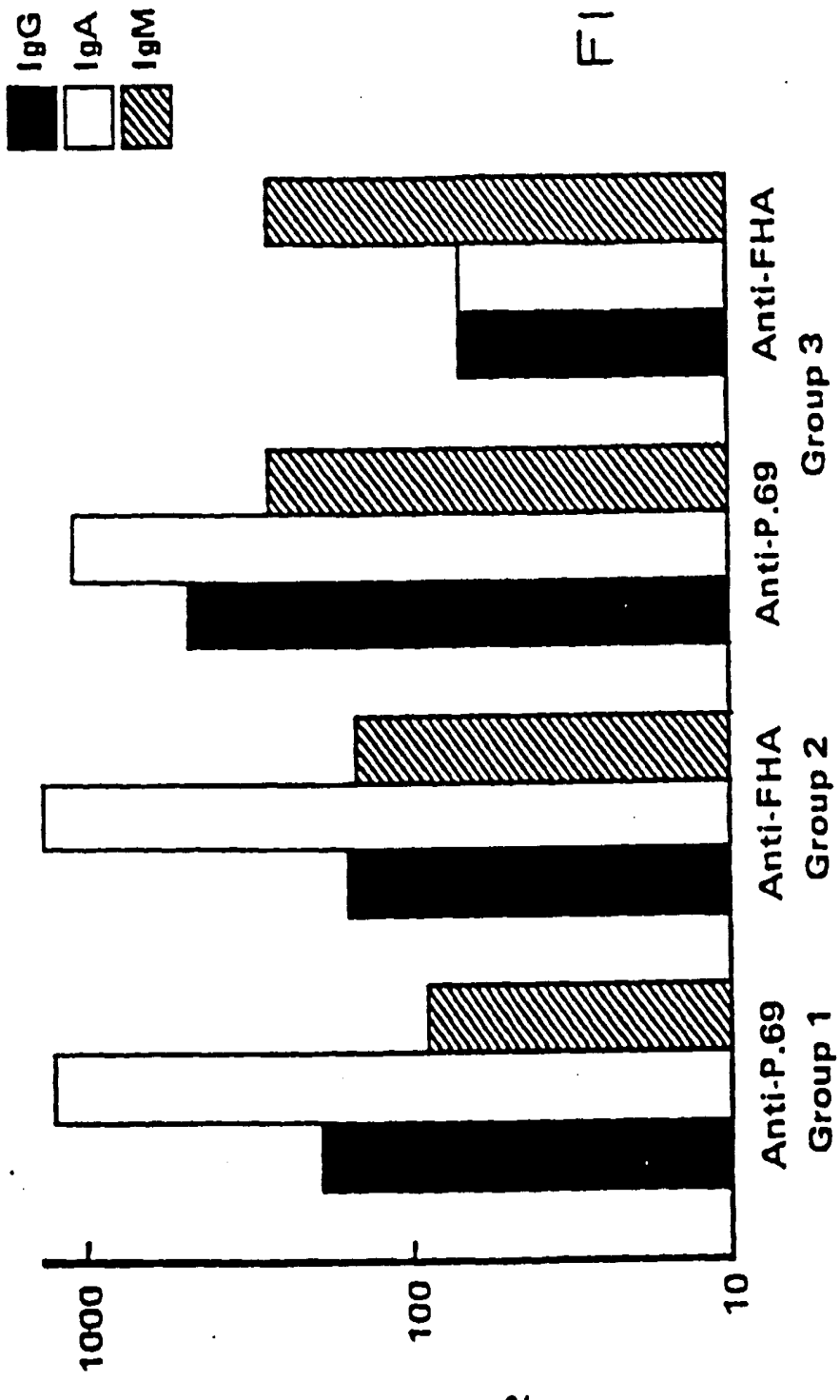

A FHA
B P.69
C OVA
100000
10000
1000
100
10
Log10 ASC/10^6 lymphocytes
IgG
IgA
IgM
4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS

FIG. 2

FIG. 3


IgG
IgA
IgM
1000
100
10
Anti-P.69
Group 1
Anti-FHA
Group 2
Anti-P.69
Group 3
Anti-FHA